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THE SIGNIFICANCE OF INTESTINAL BACTERIA FOR NUTRITION. II.

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ABSTRACT. Based on experimental work with sterile chickens the author establishes the fact that the activity of intestinal bacteria is indispensable for the life of plants and for the nutrition of vegetables and man.

In Volume XXXIV of this Archive I reported about my experimental work on /48* sterile chickens. The objective of this study was to gain further insight into the problem of the significance of intestinal bacteria for nutrition.

This study was subsequently continued over the years 1899, 1900, and 1902 and, during the Scientific Meeting in Hamburg, I had the opportunity to report on my recent results and to display a few preparations. Appropriately for the occasion, I gave a brief summary of the most important results. I was not interested at that time in presenting a comprehensive report of the experiments and of the conclusions. Much rather, it was my wish to show my preparations -- which up till then had been seen only by a small group of people -- to a larger number of my professional colleagues.

A more detailed report of this investigation is presented in this paper.

In 1898 we succeeded in keeping a chicken sterile at least up to its 17th day of life; our task for the breeding period of 1899 was to keep the experiment /49 going up to the spontaneous death of the animal.

At first I performed a few preliminary experiments for the purpose of determining how long a newly-hatched chicken could live without food, and how much weight they would lose, once without water intake and then with water intake. The animals were kept in a tall and roomy glass container open at the top; the bottom was covered with coarse, washed gravel.

The result of this experiment showed that chickens are able to survive without food for a very long time -- up to 10-12 days! However, most of the animals died after approximately 3-5 days. Intake of water seemed to be of little importance; the decisive factor was the inborn vitality. I have come to this conclusion because the external living conditions such as temperature, light, etc., were always the same, and because the absolute weight of the hatched chickens did not show any relation to the survival time. The external living conditions functioned as secondary factors: when the starving animals were kept warm and in darkness, their survival time was prolonged; on the other hand, restless running back and forth and a low external temperature exhausted their vitality very

*Note: Numbers in the margin indicate pagination in the original foreign text.

quickly. On the first and second days of life, the chickens lay stretched out on the bottom of the container almost continuously and learned very gradually to lift their head, to stand up, and to use their legs. Similarly, when life was fading, the chickens would lie motionless for days while respiration would still continue; this extended their life time -- in one case up to 12 days.

The weight loss was the same as in the sterile chickens. Animals with an initial weight of 40-45 g lost 10-15 g, so that the final weight was 30-35 g. I was not able to establish that water intake was directly related to the weight. Therefore, I have to correct my previous hypothesis that the initial weight gain /50
in sterile chickens, as well as in starving dogs, is caused by increasing water content of body tissues; the true causes of the changes in body weight will be discussed later.

In general, we have to refer the variability in the survival time of chickens kept in identical conditions to the unfortunately not measurable quantity of individual, inborn vital energy. Moreover, some races or stocks are more lively and more mobile and, therefore, they consume their vital forces faster than others. In free nature, in emergency situations when the preservation of their life is at stake, the active breeds have a certain advantage, because they are more likely to eventually find some food and thus save themselves from the death of starvation. On the other hand, the sluggish breeds have the advantage that they can tolerate greater changes in the surrounding conditions and they can wait for a longer period of time until, eventually, food is offered to them. In this way, individual and race variations balance and complement each other as far as preservation of the species is concerned.

In order to avoid the deficit in hatching eggs which occurred in our breeding experiments in 1898, the incubator was enlarged twofold so that -- instead of 80-100 eggs -- now 180-200 eggs could be incubated. This number is not very high if we consider that the eggs are used in series for the breeding experiments and that in case of a complete failure of an experimental series -- through infection of the breeding cage -- immediately a new series of fully incubated eggs has to be available so that the few months in which the experiments can be performed are used at maximum efficiency. Furthermore, it takes several days until /51
a suspected contamination of a breeding cage is confirmed bacteriologically, and then a few more days are required for the cleaning, a thorough disinfection, and the retesting of the breeding cages. The breeding cage is always filled with the fully incubated eggs on the assumption that the experiment will succeed and that the sterile chickens will live for several weeks. This makes it impossible to utilize some series of eggs, fully prepared for the experiment, not possible. The population of the poultry-yard increases while -- as the tables below show -- hardly a dozen sterile chicken are salvaged.

In addition to this, we always have to count on losses resulting from unfertilized eggs and losses resulting from unavoidable mechanical shocks occurring during disinfection of the egg shells and killing the chickens. Hence, the necessity arises of having several hundred eggs available continuously during the breeding period in order to successfully complete the experiments.

One additional unlucky accident occurs quite frequently during the actual hatching of the chicks in the breeding cage, which nature avoids by making chicks

hatch under the wings of a hen. In natural conditions, it happens very seldom that a completely mature and alive chick would die during birth, that is, when it is trying to get out of the shell. In the breeding cage, the air is more dry than under the wings of the hen, and so it happens quite often that when the chick is trying to get out of the partially broken shell, it adheres to the walls, dries out, and gets completely stuck in the shell. Its lungs cannot expand, the chick cannot breathe or it breathes insufficiently, and it suffocates within a short period of time, after about 6-12 hours. It is almost impossible to help the chicks. If we do take the risk of reopening the breeding cage, we can try to loosen the shell carefully with scissors and forceps; this almost always causes small bleedings which make the wriggling chick even more sticky. If we energetically crust the shell, then the feathers adhering to the shell tear off the skin; the chick is injured and unsuitable for the experiment. /52

This type of help can be rendered to chicks which pick through the upper part of the shell. If the lower part of the shell is picked, the accident remains undiscovered until the time when the unhatched eggs are being removed. Thus, also this mishap reduces the number of sterile chickens and increases the liability account.

All these obstacles can be overcome when the pitfalls which must be avoided are known and so, with enough patience, the experiments can be successfully performed.

In 1899 sterile breeding was successful in three experimental series: from March 28 to April 10, then from April 16 to May 15, and finally from May 25 to June 19. Two chickens were raised sterile in each of the first two series, and one chicken in the third series; all animals were observed up to the time of spontaneous death. The life span of the individual chickens ranged from 11 to 29 days, and the weight loss amounted to 36% of body weight, while in the controls body weight increased up to 154%.

For a description of the general arrangement of the experiment and of the special bacteriological precautions which were taken, the reader is referred to an earlier report in the 34th volume of this Archive. The precautionary measures described there were intensified in the subsequent years -- namely bacteriological controls of the dejections, food, water, and the air were performed in a thorough manner at the beginning, at the end, and several times during the experiment. Furthermore, the shells of the sterile, hatched chickens were not only weighed but also molded collectively in gelatin in order to be able to check for sterility at any time. Even after months, these shells did not blacken the gelatine -- an observation to which I will return later -- and therefore they can be considered not only free of germs, but also free of corrosive elements. /53

All aseptically raised chickens in this year and also in the subsequent two years were observed up to spontaneous death, and only then were they embedded in gelatine. In a few cases I postponed the imbedding for several days after death in order to be able to observe whether any postmortal changes occurred on the body. As expected, a gradual desiccation and finally mummification took place without a hint of decay (putrefaction). Such chickens could not be used as evidence of weight loss as compared to the controls. Therefore, we did not

repeat these experiments, but we imbedded the animals immediately after death had been diagnosed. It was not always possible to make this diagnosis on the basis of the appearance of the animals because the dying chickens often lay lifeless and unmoving for days. Their respiration and heart beat were reduced to such an extreme minimum that they could be detected through the glass walls of the container only with the help of good binoculars.

The behavior of the aseptically raised chickens during their life was quite interesting. As I had mentioned in my first communication, I was originally of the opinion that a hen with a few chickens of the same age should be kept next to the sterile chickens, although separated from them by a glass wall, so that the motherless animals could learn through imitation how to search for and eat food and water. It turned out that this was not necessary. The hatched chicken can usually stand on its legs on the second day. At first it staggers back and forth, and falls down again to rest for hours. But soon it learns to stand and run securely, and then it immediately begins to poke the ground with its beak /54 and to pick up small granular objects. As mentioned before, the ground consisted of washed, fine gravel mixed with food suitable for young chickens: soaked millet seeds, hard-boiled chopped eggwhite, and crushed egg shells. It was possible to see and also to determine from the analysis of the dejections that very soon the animals knew how to distinguish the various kernels, that they swallowed very little of the gravel and took chiefly the food. Similarly, they found water and avoided falling into it. During the first two days while their legs still moved clumsily, some chickens stumbled over the edge of the water container into the shallow (1 cm high) water; they immediately got up and stumbled or rolled over the edge back onto dry ground. The instinctive independence of these animals is outstanding!

Another interesting observation was the continuous hunger of the sterile chickens: they ate, digested, and eliminated dejections incessantly. This perpetual activity was much more conspicuous in the sterile chickens than in normally fed animals. Also in the latter -- as one can plainly see on any poultry-yard -- the intestinal canal possesses an enviable work capacity! But the aseptically raised animals greatly surpass in voracity and in intestinal excretory rate the normally fed controls.

The sterile chickens are also much more restless; they continuously chase around for food. When one chicken gets hold of a bit of egg shell or a kernel and cannot swallow it right away, the others immediately try obstinately to snap it away from it. It makes a great effort and swallows the booty, and then the group scatters into a new onslaught upon the sterile food on the ground.

And in spite of this continuous intake and digestion of food, the animals /55 do not grow, but their body weight and their energy diminish.

As a result of the experiments in 1899, we had five aseptically raised and spontaneously dying chickens, the longest survivor of which lived from April 16 to May 15 reaching an age of 29 days. Upon hatching this chicken weighed 51 g, and upon death 36 g; thus, during its life of 29 days, it lost 15 g or 29% of its body weight. In the meantime, the corresponding control chicken gained 77 g or 154% of its body weight. The other experimental animals displayed similar

weight losses and gains which were proportional to the life span and to the initial weight (see table below). Only chicken Nr. 5 which lived for 25 days, suffered a still greater weight loss than the one which lived for 29 days -- namely, it lost 18 g or 36% of its initial body weight, while the control chicken increased its body weight by 100%.

In 1900 we did not achieve especially good results, because I returned from my scientific travel to Bombay only at the beginning of May and, therefore, was not able to take full advantage of the breeding period. Moreover, we had an additional, unforseen mishap with one experimental series. As usual, six eggs, incubated for 19 days, were placed into the breeding cage. Unexpectedly, all six hatched and, in a few days, we had six brisk chickens jumping around in the cage. Since I considered the 1/3 square meter large bottom surface of the breeding cage too small to give the six chickens the necessary freedom of motion (in all other experiments usually two, at most three chicks hatched out of six eggs), I decided on the 6th day to divide the group by transfering three chickens into a second, newly constructed and thoroughly sterilized cage. Through this procedure the /56
glass container had to be opened several times, food and water for the separated chickens had to be resterilized and rechecked, and separate analyses of their dejections, lost feathers, etc. were performed. In short, one day the analyses showed that all samples were contaminated with a mucous mould fungus and, in this way, all six chickens were lost for our experiments.

Nevertheless, we still succeeded in 1900 in raising three sterile chickens up to their spontaneous death. The longest survivor lived for 30 days, and lost during this time 17 g or about 32% of its body weight, while the control chicken gained 62 g or 117%.

The actual goal for the year 1900 was the feeding of sterile experimental animals with certain types of bacteria. However, because of the untoward incidents and the ending of the breeding period, this project was postponed to the next year*.

In the spring of 1901 we began the investigation of the problem of whether or not feeding of intestinal bacteria to aseptically raised chickens has an effect upon their nutritive condition and life span.

The sterile breeding cage, into which the sterilized eggs would be placed for hatching, was divided completely into two compartments by means of a vertical and movable glass plate inserted between 2 cm high rails. The bacteria-tight sealing of the two compartments presented certain difficulties, because the chickens picked on every packing material and plucked it out. Therefore, cotton and /57
asbestos could not be used. Finally, we solved the difficulty by bending and compressing the free edges of the rails which were soldered to the bottom, the back wall, and the top of the container. The space in the rails was filled with asbestos wool, and when the glass plate was inserted into the compressed rails, the elastic recoil of the edges produced a tight fit, and the chickens were un-

*In September 1900 I had the pleasure of showing all my available preparations, which previously had been displayed to the Bioresearch Society in Freiburg, to professors of hygiene who, on their way to the meetings in Trier and Aachen, visited the Hygiene Institute in Freiburg.

able to get to the asbestos wool.

The front wall of the breeding cage was also divided into two parts which were framed in metal. Their central vertical edges did not meet in the midline of the cage, but were separated by a distance equivalent to the thickness of the dividing glass plate. Thus, the slit was filled with the glass plate which was later pushed in entirely, forming the dividing wall. The whole arrangement functioned very much like the light-tight closure of the photographic double-cassette during exposure of the photographic plate. The pushing of the slide plate in front of the photographic plate is equivalent to our pushing of the glass plate and dividing the breeding cage into two compartments.

The purpose of this arrangement was to let at first all the aseptically hatched chickens grow together for a relatively long time. On the 12th to 15th day, when the animals became thin and weak, I intended to insert the dividing glass plate and to supply with bacteria only the chicken(s) of one compartment, while the other side would serve as control. For this purpose, each compartment had to be equipped a priori with its own ventilation, water container, corn-bin, and thermometer. Preparations were done throughout the winter, but the acid test came with the actual experimentations. Until the defects were recognized and the apparatus improved, half of the breeding period was gone again, and only the ex- /58
periment which was begun on the 3rd of May could be utilized for the study.

On the 3rd of May 1901, four sterile chicks hatched and behaved on the subsequent days in their characteristic manner. Since, however, two of the chickens seemed very weak, we inserted the dividing glass plate already on the eighth day, closing off in each compartment one weak and one strong chicken.

In order to effect the desired bacteriological conditions, I collected a freshly eliminated dejection of an adult hen, which had grown up free, suspended it in circa 20 g broth, and poured the opaque fluid over the bottom of the cage so that the gravel, the scattered millet seeds, as well as the larger stock of sterile food lying in a back corner of the cage, were infected with the freshly suspended intestinal bacteria of the hen. The last few drops were poured into the drinking water, and then the experiment was left alone. In both compartments of the breeding cage, the chickens ate as before and displayed on the second day, the 12th of May, no particular differences or changes in their behavior. Only the weak chicken in the sterile compartment was very languid and lay for hours on the floor. On May 13, it died and in the late afternoon it was embedded in gelatine. Since the glass container had to be opened, the occasion was used to take samples of water, gravel, and millet seed from the sterile compartment for bacteriological analyses.

Fortunately, the samples passed the test; the sterile part of the cage had not been contaminated with the intestinal bacteria of the other part. The weak chicken of the septic compartment did not prosper either. Although it lived for /59
5 days longer than its sterile counterpart, it died despite the presence of the intestinal bacterial. Since it was infected, it was preserved in alcohol; otherwise it displayed nothing noteworthy in its weight or general behavior. On the other hand, the difference in the development between the two strong chickens -- the sterile one and the one with intestinal bacteria -- increased progressively.

The latter chicken grew and thrived, its feathers became smooth and bright, its movements more forceful, quiet, and certain, while the sterile animal continuously devoured food and ran in a conspicuous manner back and forth along the dividing glass plate as if it wanted to get to the other chicken. On the 20th day of May -- that is, on its 16th day of life -- it became obviously languid and on the 21st of May it expired. It lost 10 g or circa 23 1/2% of its (initial) body weight, and it was completely sterile.

The chicken fed with intestinal bacteria had now the entire cage at its disposal, where it was kept and observed until May 25; then it was transfered into the open yard to the other control hens, where it grew into a sturdy animal. Its initial weight was 46 g, and its weight at the end of the experiment, and prior to the transfer into the open yard, was 52 g. Thus, during the 14 days of bacterial feeding, the chicken gained enough weight to cover the loss of the preceding eight days of sterile feeding and, on top if it, to have a net gain of 6 g.

Control data on the sterile chickens which died on the 13th and 21st of May (see Table II) were obtained, not from the animals kept in the breeding cage and treated with intestinal bacteria, but from control chickens raised simultaneously but uncaged.

The tables do not contain data on the intestinal-bacteria-chickens and their controls, because the former do not belong to the "sterile" column. The experiment was terminated on May 25, because I did not want to have the entire apparatus occupied by one single chicken which had already yielded all the necessary data. /60

By the end of May, we expected the hatching of a new series of incubated eggs and we had to utilize the remaining six days for cleaning and disinfection of the apparatus, for the distribution of the fresh and sterile materials, and for performance of bacteriological tests.

Everything was ready on May 30, the new eggs were sterilized, eight of them were placed into the breeding cage, and on May 31 four chicks hatched. Two of the remaining eggs did not hatch at all because the chicks either died or were fatally injured by the manipulation of the eggs during disinfection. The other two chicks got stuck during the actual hatching; as we saw later, one chick lay with the head down, and the other could not loosen itself from the shell. In nature, the hen helps in such emergency situations; for reasons described above, it was not advisable for us to render assistance.

In the early spring of this year, I charged Dr. Rahner, second assistant at the Hygiene Institute, with the continuation and differentiation of the bacteriological tests of normal hen dejections which had been worked out by Dr. O. Korn. In the meantime, this work had been published in the "Centralblatt für Bakteriologie" and it showed that among the intestinal bacteria appearing early in chickens, a fission-fungus belonging to the group of the bacteria coli was the most numberous one. It is always present in the dejections of adult hens in the same proportion and in the same conditions as the bacillus coli comm. of man. In fact, the bacillus coli gallinarum is so similar to the bacillus coli hominis that, under appropriately changed breeding conditions, an absolute identity of

both races should be established. Fresh, three-day old, pure cultures of the /61
bacilius coli gallinarum were always kept in stock. The chicks which had hatched on May 31 were still quite vivacious on June 6. Since I did not want to wait for the second time until their vitality had ended, the dividing wall was inserted enclsoing two chickens in each compartment. Control samples of water, gravel, and droppings were taken, and then a broth suspension of a three-day-old agar culture of the bacillus coli gellinarum was poured over the entire floor of one compartment. Thus the experiment was begun!

On June 7 and 8 no differences or changes were observed in the four chickens. On June 9, one of the sterile chickens became visibly weak, and on June 11 it died. Since in the meantime, the second sterile chicken was prostrate and was expected to die soon, the glass container was not opened immediately to remove the first dead animal, but only on the afternoon of June 12 when the second chicken died. Both were imbedded in gelatine and remained sterile, as the available preparations demonstrate.

The chicken which served as control to the one which died on June 11, was killed on the same day and, after determination of its weight, it was preserved in alcohol.

The two chickens fed with bacterium coli gallinarum were healthy and alert, and grew considerably during the subsequent eight days. Unfortunately, due to a misunderstanding, these chickens were later set free exactly as the one from the preceding experiment with hen dejection. Correctly, the animals should have been analyzed bacteriologically to see whether their intestines contained only the bacterium coli gallinarum and then, as a further check, they should have been preserved in alcohol.

But, a new series of incubated eggs was expected to hatch in June. The /62
apparatus had to be disinfected and prepared, and at that occasion the mishap happened that the two coli-chickens, which looked exactly like the ones raised free, were taken out and placed in the poultry yard.

On top of it all, the last breeding experiment failed completely. Three chicks hatched on June 30. On July 5, when bacteria coli were distributed, test samples were taken, and their analysis revealed that a mould fungus and the yellow sarcine had colonized the breeding cage. This invalidated the experiment and, at that point, all experimental work for that year was terminated.

(See Tables I and II)

In reviewing the findings, we see that in spite of certain improvements in the arrangement of the experiments as compared to earlier breedings, we still have many difficulties to overcome in order to achieve indisputably pure experimental results.

Data on sterile chickens, summarized in Table I, show great fluctuations /63
in body weight losses without any correlation either with the initial body weight or the life span. It has been mentioned earlier that the inborn "vital energy" -- a factor which unfortunately cannot be measured quantitatively -- plays an important role. It is even more regretable that a much meaner factor affected

TABLE I
STERILLY (ASEPTICALLY) RAISED CHICKENS

Number	Year	Month	Day	Date of Death	Age In Days	Initial Weight	Final Weight	Loss
						g	g	g
1	1899	March	28	8 April	11	46	31	15
2	1899	March	28	10 April	13	45	31	14
3	1899	April	16	7 May	21	48	38	10
4	1899	April	16	15 May	29	51	36	15
5	1899	May	25	19 June	25	50	32	18
6	1900	May	18	30 May	12	46	31	15
7	1900	May	18	30 May	12	45	32	13
8	1900	May	18	17 June	30	53	36	17
9	1901	May	3	13 May	10	46	36	10
10	1901	May	3	21 May	18	43	33	10
11	1901	May	31	11 June	10	40	32	8
12	1901	May	31	12 June	11	42	32	10

TABLE II
NORMALLY FED CONTROL CHICKENS

Number	Year	Month	Day	Killed	Age In Days	Initial Weight	Final Weight	Gain
						g	g	g
1	1899	March	28	8 April	11	47	54	7
2	1899	March	28	10 April	13	46	56	10
3	1899	April	16	7 May	21	49	118	69
4	1899	April	16	15 May	29	51	128	77
5	1899	May	25	19 June	25	49	98	49
6	1900	May	18	30 May	12	47	54	7
7	1900	May	18	30 May	12	46	54	8
8	1900	May	18	17 June	30	53	115	62
9	1901	May	3	13 May	10	48	54	6
10	1901	May	3	21 May	18	45	56	11
11	1901	May	31	11 June	10	39	43	4
12	1901	May	31	12 June	11	45	53	8

the final weight of the chickens: whether they died with a full or an empty crop. If prior to death the chicken thoroughly fills its crop, it may become 5 g heavier than a similar animal which, however, did not have the strength just prior to death to seemingly satisfy its perpetual hunger by a final fill of its crop. One could possible make a cut in the crop and empty it before embedding the animal in gelatine. This is a questionable procedure if we consider the care and speed with which the animals are conserved. In any case, this was not done, and among our preparations we see animals with more or less slim necks as well /64
as animals with bulging crops; in the tables, the undigested content of the crops is contained in the figures representing the body weight of the animals.

This is an error of measurement which has to be overcome.

Another discrepancy results from the fact that the control chickens raised free do not develop in exactly the same way as those raised naturally by a hen and protected by her during their first few weeks of life. With the mother around, the chickens rest every day for several hours in uniformly warm and humid conditions under the protective wings of the hen. Their loss of heat and energy decreases, and they become better prepared for the utilization of new food. On the other hand, the incubator chick, even when they are raised with the greatest care, are much more restless and they continuously run and jump around all over the poultry-yard. Therefore, they do not develop as fast, that is, they do not gain weight as fast as the chickens under the hen. A hen does not easily accept chickens which are not her own; she picks on them and chases them away from her own flock. Fortunately, this circumstance affects both of our groups equally, the sterile and the normally-fed chickens. Therefore, on a comparative basis, the results remain unbiased. Nevertheless, the control data in Table II do not quite match the natural values, but are smaller.

In order to achieve better breeding results, I would recommend that, in addition to the incubator, several turkey-hens sit on hen eggs. This animal constitutes an extremely reliable breeding apparatus and it can breed up to 30 eggs at once. The incubator requires laborious monitoring, the necessary daily /65
roll-overs injure some eggs, and if the apparatus fails, the entire set of 200 eggs may be lost.

Finally, I should like to recommend that the movable glass plate not be used which divides the breeding cage into sterile and bacteria-fed compartments. It would be better to set up two breeding cages and let the sterile eggs hatch separately in each cage. The bacteria-tight separation of the two halves was very unreliable. We would have advanced much farther with our bacteria-feeding experiments if we did not have to repair the seal of the dividing wall so often.

Objections can be raised to the technical imperfections, and removal of the sources of errors can be stressed, but there are no doubts that the following principle is correct: the activity of intestinal bacteria is indispensable for the nutrition of animals -- especially of warm-blooded vertebrates.

This view is supported not only by general scientific considerations, but above all by the experimental results of our breeding of sterile chickens.

The principle is also in agreement with the findings of other investigators,* even with those of Nuttal-Thierfelder, as I demonstrated in my previous communications.

Recently Mme. O. Metschnikoff** successfully completed her tedious work of breeding sterile frog larvae (which developed from aseptic eggs) for 63 days. At the end of the experiment, the larvae fed aseptically with bread had an average weight of 25 mg and a length of 15.5 mm, while the non-sterile, but otherwise identically maintained, control larvae had an average weight of 142 mg and a length of 26.5 mm.

Mme. O. Metschnikoff justly concluded that the bacteria were necessary for the growth of frog larvae; she intends to continue the experiments.

The results of my experiments on sterile breeding of chickens have shown that the same principle applies to the development of hens. Mme. O. Metschnikoff had certain doubts as to the conclusiveness of my earlier experiments, suspecting that the disinfection of the incubated eggs with mercuric chloride affected the resistance or the vitality of the hatched chicks. These doubts should be resolved by the fact that the action of mercuric chloride is very superficial; it could not have penetrated through the egg shell several millimeters deep without producing visible changes in the shell. Furthermore, the presence of even trace amounts of the mercuric chloride in the sterile, incubated eggs and the egg shells embedded in gelatine always becomes evident in form of a black-brown zone which forms in the gelatine around the egg, probably through reduction of the mecury. We do have a large number of such control eggs and shells embedded in /67
gelatine; all of them belong to the first phase of our experiments. In subsequent work, every tract of mecuric chloride was completely removed through repeated scrubbing and washing of the eggs with saline followed by dry-wiping with

*Levin, who had the opportunity to accompany the Natthorst expedition in the summer of 1898, established the hypothesis (Annales de l'Institut Pasteur, Vol. XIII) that in arctic zones the intestinal content of most warm-blooded animals was absolutely free of bacteria. We should like to comment that polar birds as well as polar mammals stay in the ice regions for limited periods of time, and are otherwise migrating animals who move in search of food. Their food -- so far as they do not eat up each other -- consists of higher aquatic animals: fishes, crustaceans etc. These, in turn, depend on smaller animals which live in warmer sea currents and which are not at all bacteria-free but contain low organisms of many varieties and in large quantities, as has been demonstrated by numerous studies, especially by those of Nordenskiöld and Nansen. Although for these reasons alone it should be obvious that the intestinal content of arctic animals cannot be free of bacteria, we also have positive findings of /66
other investigators, all of them pointing to the same fact. H. Chauveau, for example, participated in 1900 in the expedition of the Prince of Monaco especially for the purpose of re-examining Levin's data. Contrary to Levin's findings, and without a single exception, he proved the presence of bacteria in the intestinal content of seals, foxes, and numerous types of arctic birds.

**Annales de l'Institut Pasteur, Vol. XV, p. 631.

sterile cotton. In the last few years, eggs disinfected with mercuric chloride were also treated with potassium sulphide which chemically decomposed the mercuric chloride.

We should like to point out that any effects of the mercuric chloride should also be visible on the control chickens, since their eggs were treated in the same manner as those of the sterile chickens. Since the control animals thrived well -- at least they gained weight in a normal way -- it follows that either the mercuric chloride had no negative effects or, if it had, the effects applied equally to the sterile and the control chickens. Therefore, our conclusions, which are based on the relative difference in the developments between the two groups, are valid.

Most certainly, one should be careful with generalizations, that is, one should not conclude from the specific nutritive requirements in one species that the same requirements are true in another species. However, Mme O. Metschnikoff is going too far when she desires to explain the difference between the results of Nuttall-Thierfelder experiments on guinea-pigs and my experiments on chickens on the basis of various bacterial requirements of these two different animal species. The nutritive conditions of warm-blooded vertebrates are too similar to presuppose such a fundamental difference between the nutrition of hens and that of guinea pigs. On the other hand, I would not proceed with generalizations as far as to make a comparison between the nutrition of mites and that of higher vertebrates. Most probably Mme. Metschnikoff was referring to the sucking mites, specifically the ticks, when she reported that lower organisms were not harmful to them but, to the contrary, served them as food. Such a relationship occurs /68 also in higher vertebrates and in man. For example, with new wine large quantities of yeast fungus are conveyed into the human stomach, and they are digested and serve the body as food, exactly as a living oyster is digested and becomes food. The tremendous masses of bacteria which are killed by the warm-blooded animal body owing to its high temperature -- this is a powerful protective mechanism in the fight against lower organisms -- are all decomposed by the digestive juices, and there is no reason to believe that the peptonized mycoprotein could not be taken in and processed and asimilated by the body.

Of what significance this processing may be, we cannot answer at present. If there are really as many different "stuffs" circulating in body juices and tissues as are quoted by names, then they must be replenished from sources, among which the intestinal content is the first to be taken into consideration.

The fact is that the results of my experiments do not contradict Nuttals' and Thierfelder's published findings about sterile feeding of guinea pigs and, as should be obvious from the arrangement of my experiments, I was actually stimulated to this research by the experiments of Nuttal and Thierfelder. I was of a different opinion only in the interpretation of the results and, in order to have positive supporting evidence, I decided to perform experiments on chicken eggs.

With my present experience, I consider it probable that the Nuttall-Thierfelder experiments could be resumed and successfully continued. However, these experiments can be useful for the theory of nutrition, that is, for the analysis

of the significance of the intestinal bacteria only when, instead of milk feeding, the guinea pigs receive their normal plant food.

The milk, which in the case of chickens is analogous to the chicken protein /69
in the egg, constitutes a part of the mother's organisms pre-allotted for the young offspring. As long as the young animal is dependent on the function of the maternal body, it cannot be considered independent in its subsistance, exactly as we cannot speak of an independent subsistence of a chicken which is still in the egg absorbing maternal egg white.

The significance of the intestinal bacteria becomes a problem only when the individual, which may be a chicken, a guinea pig, any other animal, or man, has to keep alive independently of the maternal organism. All these experiments, which undoubtedly are very meaningful, would be brought to a completely satisfactory conclusion if we succeeded in confirming the necessity of the intestinal bacteria for proper nutrition through experiments on mammals.

For the performance of these experiments on guinea pigs, considerable external resources would have to be available which the Hygiene Institute does not have at its disposal at present. Therefore, for the time being, experiments on aseptically raised chickens will have to serve as the basis for further investigation of our problem.

The first objective is to have duplicate experiments with the pure bacillus coli gallinarum, that is, to keep chickens, which contain exclusively the bacillus coli gallinarum, up to a very advanced stage of growth. The degree of the development of these chickens should be compared not only with the aseptically raised animals, but also with the control chickens raised free. Of all the bacteria which constantly inhabit the intestines of chickens, at least the most important ones should be analyzed for their individual and combined effects. This requires a joint histological and chemical investigation of the aseptically raised and bacteria-fed animals. Furthermore, the chicken is a good experimental subject for the study of a number of pathogenic bacteria of the intestines, and /70
it is probable that such experiments would yield valuable information about the pathology of the intestinal canal.

In which way, if at all, the results of this investigation could be utilized for a better understanding of human pathology, will be resolved in the future. It is by no means improbable that a better knowledge of normal biological processes inside the intestinal canal would advance our understanding of the physiology of nutrition and of the pathology of the intestinal canal, and would more successfully clarify the pathogenesis of some intestinal diseases.

It is well known that our German "plague" is not the bubonic plague, but typhoid fever. Since none of the applied remedies has succeeded in preventing perenial outbreaks of serious typhoid epidemics, no path should remain uninvestigated which could throw some light on the obscure relation between the typhoid bacillus and the bacillus coli. The road from the physiological effects of the bacillus coli gallinarum in the chicken intestines to the successful combat of the typhoid bacillus in the human intestinal canal may be very long. However, it is the only one which can clarify the physiological and the pathological

processes in the intestinal tract and, therefore, sooner or later, this road must be entered upon, and it will lead to the goal.

For the present time, it has been established that the activity of intestinal bacteria is indispensable for the life of plants and for the nutrition of vertebrates and of man.

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